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Electronic Biology and Cancer

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Biology today is a molecular science. Its charm is in the wonderful subtlety of its reactions, the main actors of which are the protein molecules. It is difficult to believe that this subtlety could be brought about by clumsy macromolecules without the participation of much smaller and more mobile and reactive units which could hardly be anything but specially reactive electrons. One may wonder to what degree protein molecules are the actors, or the stage for the drama of life, and to what degree life itself is a molecular or electronic phenomenon. Such a basic problem can be approached only with the broadest philosophical outlook, beginning at the origin.

Life originated on a dark and airless globe, covered by dense water vapor. Having condensed from hydrogen, the atmosphere had to be strongly reducing, dominated by electron donors, reducing substances which tended to give off rather than take up electrons. At the resulting high electronic pressure, all allowed places on the energy bands of the protein molecule had to be occupied by electrons, leaving no room for mobility, making the protein dielectric, an insulator. The single orbitals had to be occupied by pairs of electrons which, spinning in opposite directions, compensated each others magnetic moments. The protein had to consist of well-balanced, stable, dielectric, closed-shell molecules, which had no unbalanced forces that could link them together to complex structures.

We can only philosophize that under such inhospitable conditions life could build only simple systems which performed the most basic vegetative functions that demanded no complex structures: fermentation and proliferation. Fermentation produced energy and proliferation made life perennial, proceeding as fast as conditions permitted.

This first, dark and anaerobic period I call the "α period," and the corresponding state of living systems the "α state." When, owing to the cooling of our globe, its water envelope condensed and light could reach its surface, life began to develop and differentiate. It used the energy of light to separate the elements of water, H and O. The hydrogen it fixed by linking it to carbon, creating foodstuffs. The oxygen it released into the atmosphere. Oxygen is a powerful oxidizing agent, an electron acceptor, and so, henceforth, life was no longer dominated solely by electron donors, but became dependent on the quotient D/A, the relation of donors to acceptors. Oxygen can oxidize, take electrons from other substances. It can also take electrons from protein. By taking electrons from energy bands it could make room for motion, transforming the protein from an insulator into a semiconductor. By taking single electrons it could uncouple electron pairs which upsets the balance, making reactive free radicals from the inert molecules, a "free radical" being a molecule containing an unpaired electron which can be detected by the signal it gives in the electron spin resonance (ESR) spectroscope. With their transformed proteins and their unbalanced forces, the living systems started to differentiate, to build increasingly complex structures with increasingly complex functions. This second period of life, which followed the appearance of light and oxygen I will call the "β period," and the corresponding physical state the "β state."

If it were actually the oxygen that initiated the transformation from the α to the β state by taking electrons from protein, then this transfer of electrons would have to be one of the most basic processes of biology. Its regulation will be my central theme.

OXYGEN AND DICARBONYLS

Electrons, in molecules, are paired and tend to go from one molecule to the other pairwise. Thus bivalent acceptors, which can take up two electrons, will always tend to take over two electrons, even if the two go over one by one. The transfer of an electron pair to oxygen is called "*oxidation*", "*burning*." This transfer of an electron pair leaves a somewhat smaller, but still well-balanced molecule behind.

The situation will be different if the acceptor is monovalent, can take up but one electron. Taking one electron means that both molecules, the one which accepted the single electron and the one which donated it, become reactive free radicals. The transfer of a single electron from one molecule to another is "*charge transfer,*" which was hitherto looked upon by most biologists as a rare event, an oddity, an item of Nature's own curiosity shop.

The oxygen molecule, O=O, consists of two O atoms linked together by a double bond. If the double bond opens up, —O—O—, a bivalent electron acceptor is produced. If the —O—O— breaks up into single O atoms, these atoms again are bivalent, so the oxygen will tend to burn, take up electrons pairwise without creating free radicals. So oxygen, as such, could not transform the protein into free radicals. Nature solved this problem by linking oxygen atoms to carbon, C atoms, instead of linking them to one another. An oxygen linked to carbon by a double bond is C=O, a carbonyl. Carbonyls are monovalent acceptors. They are weak acceptors because the C=O group is too small to accommodate easily a whole additional electron. However, if two carbonyls are linked together to a dicarbonyl, then the π electronic systems of the neighboring (conjugated) double bonds fuse to a big π system, which easily takes up a whole electron, is a "strong" acceptor, but is, all the same, still a monovalent.

The simplest dicarbonyl is glyoxal, the first methyl derivative of which is methylglyoxal (Fig. 1). This makes our problem very exciting because more than 60 years ago two Englishmen and a German, H. D. Dakin and H. W. Dudley (1913) and C. Neuberg (1913), discovered a most active enzymatic system present in all living cells, which can transform the reactive methylglyoxal into an inactive D-lactic acid with amazing speed. This enzymatic system is called "glyoxalase." Nature does not indulge in luxuries, and if there is such a most active and widely spread enzymatic system, it must have something very important to do, but nobody knew what, because neither methylglyoxal, nor D-lactic acid were known as metabolites. Could, then, methylglyoxal have been responsible for transforming the proteins into conductors and free radicals by taking electrons out of them? Glyoxalase could act as its antagonist which prevents these changes by inactivating it. Methylglyoxal and the glyoxalase, together, could then be one of the most important tools of cellular regulation, one being the green, the other the red light.

Einstein said that Nature is simple but subtle. The methylglyoxal molecule is very simple, but has, all the same, very specific characteristics. The calculations of Alberte Pullman showed that it has a very low lying empty orbital (personal communication) which makes it a strong acceptor. This orbital has to be on the ketonic oxygen, while the aldehydic group could serve to establish a link with

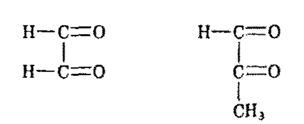

Fig. 1. Glyoxal and methylglyoxal.

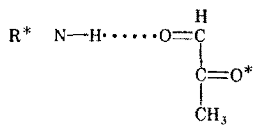

Fig. 2. H-bond between a peptide and methylglyoxal. The unpaired electron is symbolized by an asterisk. R stands for the peptide bond.

the protein. Linus Pauling showed, many years ago (1959) that the NH of proteins readily forms hydrogen bonds with carbonyls. In Fig. 2 the peptide bond of protein is symbolized by R. When its electron goes over to the ketonic group of methylglyoxal (marked by an asterisk), it has to pass six atoms, and an H-bond will thus be very far from its earlier pair, left behind on R. This is very important because it was thought that two radicals, formed by charge transfer. must dissociate to have their electrons uncoupled to give an ESR signal. In the case of methylglyoxal, however, the charge transfer complex can be expected to be uncoupled and give an ESR signal without dissociation. There is no need to suppose that protein and methylglyoxal dissociate after the electron transfer; the final product of the reaction can be a biradical, consisting of the positively charged protein and the negatively charged methylglyoxal radical. It is even difficult to see what could drive the two apart since the forces holding them together are only increased by the charge transfer energy and the attraction of the opposite charges.

AMINES AND DICARBONYLS

Proteins are very complicated substances, so before addressing myself to them I tried to collect some data by studying the interaction of simpler amines and methylglyoxal. The simplest amines are methyl and ethylamine, CH_3NH_2 and $CH_3CH_2NH_2$. I started with mixing a watery solution of these amines with the solution of methylglyoxal, expecting them to react and produce the vivid colors of free radicals. They slowly formed a so-called "Schiff base," and no radicals. Only the weak yellow color of the Schiff base developed which gave an absorption at the short wavelength end of the spectrum. Either no free radicals were formed, or else they were too unstable to accumulate and give visible colors. So I repeated the experiment using dimethylsulfoxide as solvent which, in my experience, stabilized free radicals. An intense red color developed, and in the spectroscope the solution gave two sharp peaks, one at 315 and another at 475 nm, and gave a spin resonance signal in Dr. H. Kon's ESR spectroscope (Kon and Szent-Györgyi, 1945), which indicated that a whole

electron was passed from amine to carbonyls, leading to the formation of free radicals. By the addition of an amine, the dicarbonyl could thus be readily made into a reactive radical.

With this experience in hand I mixed protein with methylglyoxal, expecting colored radicals to be formed, but nothing happened, the dicarbonyl did not interact with protein at all, it could not bind to it. But if the free methylglyoxal did not bind to protein, perhaps the biradical it formed with amines did? When brought together with this biradical, the colorless protein changed into a dark brown substance which had the same color as the proteins which build the structure of the liver cells. Evidently, the biradical could bind to protein and take electrons from it, and so our next question was whether this protein–amine–dicarbonyl complex could also link with oxygen and pass on the electrons of protein to it. The complexes of the simple amines could not answer this question, but here an earlier experiment, performed with Jane McLaughlin, came to our aid (Szent-Györgyi and McLaughlin, 1975). We found that dicarbonyls readily form charge transfer complexes with biogenic amines, such as dopamine or serotonin, and these complexes turned black when forming charge transfer complexes with oxygen. We could thus see whether they interacted with oxygen. These dicarbonyl complexes of biogenic amines also readily complexed with protein, and could thus transfer electrons taken from it to O_2. These expectations could readily be tested in the experiment by incubating, for a short while (30 min), protein in the presence of biogenic amine, dicarbonyls, and O_2 (air): The result was a black protein complex which gave an ESR signal, indicative of a multiradical. The black color inseparable from the protein by reprecipitation was thus the color of the protein charge transfer complex in which the electrons of the protein were transferred to O_2. We can thus piece the story together and say that a bridge can be built of free radicals between protein and oxygen through which oxygen can desaturate the protein, make it into a free radical and semiconductor.

If the oxygen molecule O=O, enters into a complex with the dicarbonyl, it has to form a peroxide. That peroxides were actually formed could be shown by repeating the experiment with addition of peroxidase, which activates peroxides. In its presence the protein colored faster and stronger, indicating that the enzyme activated the peroxide, enabled it to accept a second electron. With two electrons, O=O forms (in the presence of water) hydrogen peroxide which then has to be decomposed in tissue by catalase. For the completion of the reaction, both catalase and peroxidase are needed. This gives meaning to the discovery of D. Keilin and E. F. Hartree, made 30 years ago (1945), that catalase has both these enzymatic activities. We can thus sum up the whole story by saying that protein can be transformed by passing its electrons to oxygen through a chain built of free radicals, all reactions involved being electronic and not molecular.

THE α-β TRANSFORMATION AND CANCER

To be able to answer the question whether all this has anything to do with cancer, I will have to return briefly to the α-β transformation, the profound change in the nature of living systems which followed the appearance of oxygen. As discussed before, when light and oxygen appeared, life began to develop, differentiate and build increasingly complex structures that performed increasingly complex functions. The growing complexity was incompatible with unbridled proliferation. Proliferation had to be arrested and subjected to regulation to maintain the harmony of the whole. It was arrested by two factors. The dicarbonyls inactivated the SH groups which are indispensible for cell division, forming hemimercaptals with them. It has been shown by L. Egyud and myself (1966a,b) that dicarbonyls arrest cell division reversibly in low concentration on the ribosomal level. The arrest can be eliminated by glyoxalase which decomposes the dicarbonyls and starts up cell division again.

The other factor which must have helped to arrest cell division was the semisolid structures built in the β period. Cell division involves a complete rearrangement of the cellular interior, which is impossible in a rigid, solid structure. To be able to divide, the cell has to dismount its structures to a great extent. The most striking example of this demolition is the dissolution of the cellular nucleus, the membrane of which is dissolved, and the chromatin of which is condensed to mobile chromosomes. The mitochondria are also partly dissolved, forcing the cell to derive its energy to a greater extent from fermentation, which demands no structure. So, in division, the cell dedifferentiates, dissolving its structure, going through the α-β transformation in reverse, returning to its proliferative fermentative α state.

What lends validity and major medical interest to these relations is that after it has completed its division, the cell has to find its way back to the resting β state, build up its free radical electron transport chain and structures again. Should it be unable to do so, it has to persist in the proliferative α state, continue to proliferate when no proliferation is needed, and a tumor has to result. The cancer cell is a cell stuck in the α state. The same will be the result if, for any reason, the β state becomes unstable and the cell is unable to maintain its electron transport chain. The free radicals building this chain, are colored, so their absence should declare itself by the lack of color. This suggested a comparison of the color of the structural proteins of normal tissues with those of cancer. Such a comparison has value only if we compare cancer with the homologous normal tissue. Owing to the kindness of Dr. G. Weber, I am in possession of a rapidly growing parenchymal liver tumor (Morris Hepatoma 3924A). The structural proteins of the normal liver were chocolate brown, those of cancer light yellow-green. That the difference was due to the lack of electron transport chain could be shown by adding an electron acceptor to the tumor proteins, whereupon they assumed the color of the corresponding preparation of the normal liver.

I want to conclude this paper by answering three questions.

Question I: How was all this overlooked before? The explanation is simple. The protein chemist needs crystals, and to produce them he needs protein solutions. So what he did was to extract the soluble proteins from the organism and called the extracted tissue "the residue" and sent it down the drain. As I have shown, the soluble proteins perform only the simplest vegetative functions that need no free radicals or conduction. These free radicals and conduction are needed only by the structures, but the structures cannot be crystallized and so have been disregarded by the chemist.

Question II: How does all this relate to viruses? Cell division and its regulation demands a complex chemical mechanism. Viruses cannot build such a mechanism. They can only disturb it and set proliferation going by interfering with regulation. The regulatory mechanism can be disturbed by an endless number of factors. Viruses are one of them. The cancer problem is much more complex than "virus or no virus."

Question III: Will my findings lead to a cure for cancer? They may or may not. What can be said with certainty is what Bernal told us: "That we can control only what we understand."

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